

WHAT IS CLAIMED:

1. A thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when
5 sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.
- 10 2. A thermostable ligase according to claim 1, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a
15 mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.
3. A thermostable ligase according to claim 2, wherein, in the presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a
20 pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.
4. A thermostable ligase according to claim 3, wherein the
25 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.
5. A thermostable ligase according to claim 1, wherein, in the presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than
30 wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a

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mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

6. A thermostable ligase according to claim 5, wherein the
5 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

7. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif
10 where X is any amino acid.

8. A thermostable ligase according to claim 1, wherein the
thermostable ligase has a molecular weight of 78 to 81 kDa determined by SDS-
PAGE.
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9. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

10. A thermostable ligase having 50 fold higher fidelity than T4
20 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

11. A thermostable ligase according to claim 10, wherein, in the
25 presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation
30 junction at the base immediately adjacent to the ligation junction.

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12. A thermostable ligase according to claim 11, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

5 13. A thermostable ligase according to claim 10, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

10 14. A thermostable ligase having an arginine adjacent its active KXDG motif where X is any amino acid.

15 15. A thermostable ligase having, in the presence of a Mn^{2+} cofactor, a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

20 16. An isolated DNA molecule encoding a thermostable ligase, wherein the thermostable ligase has a 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.

25 17. An isolated DNA molecule according to claim 16, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

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